Research Article

Implementation of Quality by Design for the Development and Validation of Pioglitazone Hydrochloride by RP-UPLC with Application to Formulated Forms

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Received 30 September 2012; Accepted 6 November 2012

Academic Editors: E. Boselli, M. C. Bruzzoniti, B. Carbonnier, A. Sanches Silva, and S. Valsecchi

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Quality by Design (QbD) is a philosophy that refines the level of knowledge associated with a product that uses process understanding to deliver a product with the desired critical quality attributes. The objective of this study was to develop an integrated multivariate QbD approach to develop and quantify the constituent concentrations of pioglitazone hydrochloride (PGZ) drug in its pure and formulated forms. To facilitate studies investigating the determination of PGZ in bulk drug and its pharmaceutical formulations, a rapid UPLC method was developed and validated for the determination of PGZ accompanied by its degradation studies in different stress conditions. The method fulfilled validation criteria and was shown to be sensitive, with limits of detection (LOD) and quantitation (LOQ) of 0.01 and 0.05 $\mu\text{g mL}^{-1}$, respectively. The percent relative standard deviations for robustness and ruggedness were observed within the range of 0.1–1.74. The calibration graph was linear in the range of 0.05–300 $\mu\text{g mL}^{-1}$. The applicability of the method was shown by analysis of formulated drug samples and spiked human urine. The proposed method can be used for routine analysis in quality controlled laboratories for its bulk and formulated product and this is the first reported UPLC method for the assay of PGZ.

1. Introduction

Quality by design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [1, 2]. The objective of the QbD initiative is to demonstrate both understanding and control of pharmaceutical processes to deliver high quality pharmaceutical products while affording opportunities for continuous improvement. QbD delivers a better understanding of method capabilities and limitations and ensures a superior chance of successful downstream method validation and transfer. It has become an important paradigm in the pharmaceutical industry since its introduction by the US Food and Drug Administration [3–7]. The QbD concept can be extended to analytical methods [8–15]. The analytical methods used for the analysis of active

pharmaceutical ingredients (API) and drug products are an integral part of the QbD.

Pioglitazone (PGZ), chemically known as [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-] thiazolidinedione monohydrochloride (Figure 1) is an oral antihyperglycemic [16] agent used for the action to treat diabetes. It belongs to thiazolidinediones (TZDs), also known as glitazones, which are a relatively new class of oral hypoglycemic, insulin-sensitizing drugs that are used clinically as third-line agents in the management of diabetes mellitus type 2 as they help restore peripheral insulin sensitivity. TZDs activate peroxisome proliferator-activated receptor gamma (PPAR- γ), a nuclear receptor that regulates the production of proteins involved in glucose and lipid homeostasis, and, to a lesser extent, PPAR- γ [17]. It increases systemic insulin sensitivity in tissues of animal models and humans with type 2 diabetes and the metabolic syndrome [18].

FIGURE 1: Structure of pioglitazone HCl.

PGZ is included in the United States Pharmacopoeia [19] and official drafts of European Pharmacopoeia [20], both recommend HPLC method for its assay. In the literature, several HPLC methods [21–28, 30–37], [29] are available for the determination of PGZ in, dosage forms [21–31], urine [32], plasma [33–35], and related substances [36]. Reported methods for the determination of PGZ in pharmaceuticals include HPTLC [38], spectrophotometry [39–41], electrochemical [42], and mass spectra [43].

According to literature survey, there are quite a few publications on UPLC method development [44, 45] strategy. The reported UPLC methods for the determination of PGZ are for combination drugs only [46, 47]. Method development approach with RP-UPLC specifically focused on pharmaceutical development in a QbD environment for PGZ has not been reported anywhere. Therefore, there is an unmet need to investigate a systematic UPLC method development approach for pharmaceutical development using QbD principles to ensure the quality of the method throughout the product lifecycle.

The primary objective of this study was to implement QbD approach to develop and validate an RP-UPLC method that could separate drug in the bulk and formulated forms from its potential related substances and to establish an in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product with a suitable degradation data.

2. Methods

2.1. Materials and Reagents. Pure active ingredient sample of PGZ was kindly supplied by Glenmark Pharmaceuticals, Mumbai, India, as gift. PGZ-containing tablets; Neoglit-30 (30 mg) (Novus Life Sciences Private Limited, Mumbai), Oglo-15 (15 mg) (Panacea biotec, Mumbai) were procured from the local market. HPLC grade acetonitrile was purchased from Merck, potassium dihydrogenorthophosphate, triethylamine and orthophosphoric acid were from Qualigens-India. Doubly distilled water was used throughout the investigation.

2.2. Chromatographic Conditions and Equipments. Analyses were carried out on a Waters Aquity UPLC with Tunable UV (TUV) detector. The output signal was monitored and processed using Empower software. The chromatographic column used was Acquity UPLC BEH C-18 (100 × 2.1) mm and 1.7 μ m particle size. Isocratic elution process was adopted throughout the analysis. Mobile phase used was 20:80

(acetonitrile : buffer) v/v (buffer-2.2 g. potassium dihydrogen orthophosphate in 1 litre water and 1 mL triethylamine, then adjusted the pH to 3.2 with diluted o-phosphoric acid).

2.3. Instrumental Parameters. The isocratic flow rate of mobile phase was maintained at 0.20 mL min⁻¹. The column temperature was adjusted to 25°C. The injection volume was 2 μ L. Eluted sample was monitored at 220 nm and the run time was 5.0 min. The retention time of the sample was about 2.1 min.

2.4. Stress Study. All stress decomposition studies were performed at an initial drug concentration of 200 μ g mL⁻¹ in mobile phase. Acid hydrolysis was performed in 1 M HCl at 80°C for 2 h. The study in alkaline condition was carried out in 1.0 M NaOH at 80°C for 3 h. The study in neutral condition was carried out in water at 80°C for 3 h. Oxidative studies were carried out at 80°C and 5% hydrogen peroxide for 2 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber [48]. Additionally, the drug powder was exposed to dry heat at 105°C for 2 h. Samples were withdrawn at appropriate time and subjected to UPLC analysis after suitable neutralisation and dilution.

2.5. Preparation of Stock Solution. A stock standard solution of PGZ (1000 μ g mL⁻¹) was prepared in mobile phase and used for validation.

2.6. Procedures

2.6.1. Procedure for Preparation of Calibration Curve. Working solutions containing 0.05–300 μ g mL⁻¹ PGZ were prepared by serial dilutions of aliquots of the stock solution. Aliquots of 2 μ L were injected (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of PGZ in μ g mL⁻¹ was plotted. Alternatively, the corresponding regression equation was derived using mean peak area-concentration data and the concentration of the unknown was computed from the regression equation.

2.6.2. Preparation of Tablet Extracts and Assay Procedure. Fifty Oglo-15 tablets (each tablet contained 15.0 mg PGZ) and twenty five Neoglit-30 (each tablet contained 30.0 mg PGZ) were weighed and powdered. Tablet powder equivalent to 20 mg of PGZ was transferred in to 100 mL volumetric flasks and 60 mL of the mobile phase were added. The solution sonicated for 20 min to achieve complete dissolution of PGZ, made up to the mark with mobile phase and then filtered through 0.22 μ m nylon membrane filter. The solution (200 μ g mL⁻¹ in PGZ) obtained was analysed.

2.6.3. Procedure for the Analysis of Placebo Blank and Synthetic Mixture. A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg), and sodium alginate (10 mg) was made and its solution was prepared as

described under tablets and then subjected to analysis. A synthetic mixture was separately prepared by adding pure PGZ (100 mg) to the above and the mixture was homogenized. Prepared a solution containing $200 \mu\text{g mL}^{-1}$ of PGZ was prepared from the above and extracted as described for tablets. The extracts containing three different concentrations of PGZ were subjected to assay by following the general procedures and the percentage recovery of PGZ was evaluated.

2.6.4. Procedure for Method Validation

Accuracy and Precision. To determine the accuracy and intra-day precision, pure PGZ solutions at three different concentrations were analyzed in seven replicates during the same day. The same procedure was followed in different days for the inter day precision. Mobile phase was injected as blank solution before sample injection and the RSD (%) values of peak area and retention time were calculated.

Limits of Detection (LOD) and Quantification (LOQ). The LOD and LOQ were obtained by signal to noise (S/N) ratio method [49]. LOQ and LOD were obtained by a series of dilutions of the PGZ stock solution. Precision study was performed at LOQ level also. LOQ solution was injected seven times ($n = 7$) and calculated the % RSD values for the obtained peak area and retention time.

Linearity. Linearity solutions were prepared from LOQ level to 150% of the actual sample concentration ($200 \mu\text{g mL}^{-1}$ PGZ). A total of six concentrations of the solutions were made separately and injected (LOQ, 50, 100, 150, 200, 250 and $300 \mu\text{g mL}^{-1}$ levels).

Robustness and Ruggedness. To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase ($0.2 \pm 0.02 \text{ mL min}^{-1}$), column oven temperature ($25 \pm 1^\circ\text{C}$), mobile phase composition (15:85, 25:75, acetonitrile:buffer, v/v), and detection wavelength ($220 \pm 1 \text{ nm}$) were the varied parameters. In each case the %RSD values were calculated for the obtained peak area and retention time. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. Three different columns of same dimensions were used for the analyses. The studies were performed on the same day and on three different days by three different analysts for three different concentrations of PGZ (triplicate injections). The area obtained from each concentration was compared with that of the optimized one. The relative standard deviation values were evaluated for each concentration.

Solution Stability and Mobile Phase Stability. Stability of PGZ solution was investigated by injecting the sample into the chromatographic system at equal interval of time. The peak area was recorded in the time intervals of 0, 12 and 24 hrs and the RSD values were calculated. The mobile phase stability was studied by injecting a freshly prepared sample solution at the same time intervals (0, 12, and 24 hours) with the

FIGURE 2: UV absorption spectra of pioglitazone HCl.

same mobile phase and RSD values of the peak areas were calculated.

3. Results

The wavelength for detection was obtained from the UV absorption spectra of the sample solution (Figure 2).

Method Development for Acquity BEH C8 Column, ($100 \times 2.1 \text{ mm}$, $1.7 \mu\text{m}$). All the trials are as shown in Table 1 and chromatograms are as shown in Figure 3.

Method Development for Acquity BEH C18, ($100 \times 2.1 \text{ mm}$, $1.7 \mu\text{m}$ Column). All the trials are as shown in Table 2 and chromatograms are as shown in Figure 4.

Method Development for C18 Using Different pH Conditions. All the trials are as shown in Table 3 and chromatograms are as shown in Figure 5.

3.1. Final Method Conditions. Column: Acquity BEH C18, ($100 \times 2.1 \text{ mm}$, $1.7 \mu\text{m}$; oven temp.: 25°C . Mobile phase: ACN:buffer (pH 3.2) (20:80% v/v); run time: 5 min. Flow rate: 0.2 mL/min ; diluent: mobile phase. Injection volume: $2 \mu\text{L}$ blank: diluent. Wavelength: 220 nm .

3.2. Validation of the Method. The described method for the assay of PGZ was validated as per the current ICH Q2 (R1) Guidelines.

3.2.1. Linearity and Sensibility. A calibration curve was obtained for PGZ from LOQ to 150% of its stock solution. A linear correlation was obtained between the mean peak area and the concentration in the range of $0.05\text{--}300 \mu\text{g mL}^{-1}$ PGZ. The LOD and LOQ values, slope (m), y -intercept (a), and their standard deviations are evaluated and presented in Table 4.

3.2.2. Accuracy and Precision. The results obtained for the evaluation of precision and accuracy of the method is compiled in Tables 5 and 6. The percent relative error which

TABLE 1: Observation and remarks of method development for Acquity BEH C8, (100 × 2.1) mm, 1.7 μ m Column.

S. No.	Trails taken	Observations	Remarks
1	ACN : buffer (pH 3.2) (20 : 80% v/v)	Asymmetrical peak with fronting and tailing	Not Satisfactory
2	ACN : water (20 : 80% v/v)	Asymmetrical peak with tailing	Not Satisfactory
3	Methanol : water (20 : 80% v/v)	Split peak	Not Satisfactory
4	Methanol : buffer (pH 3.2) (20 : 80% v/v)	Asymmetrical peak	Not Satisfactory

(a) ACN : Buffer (pH 3.2) (20 : 80% v/v)

(b) ACN : Water (20 : 80% v/v)

(c) Methanol : Water (20 : 80% v/v)

(d) Methanol : Buffer (pH 3.2) (20 : 80% v/v)

FIGURE 3: Chromatograms obtained for method development using Acquity BEH C18, (50 × 2.1) mm, 1.7 μ m column.TABLE 2: Observation and remarks of method development for Acquity BEH C18, (100 × 2.1) mm, 1.7 μ m column.

S. No.	Trails taken	Observations	Remarks
1	ACN : buffer (pH 3.2) (20 : 80% v/v)	Symmetrical peak	Satisfactory
2	ACN : water (20 : 80% v/v)	Asymmetrical peak with tailing and early elution	Not Satisfactory
3	Methanol : water (20 : 80% v/v)	Very broad peak	Not Satisfactory
4	Methanol : buffer (pH 3.2) (20 : 80% v/v)	Broad peak	Not Satisfactory

TABLE 3: Observation and remarks of method development for different pH conditions with Acquity BEH C18, (100 × 2.1) mm, 1.7 μ m column.

S. No.	Trails taken	Observations	Remarks	Theoretical plates	Tailing factor
1	ACN : buffer (pH 3.2) (20 : 80% v/v)	Peaks found symmetrical	Satisfactory	3330	1.02
2	ACN : buffer (pH 4) (20 : 80% v/v)	Peak eluted early with less theoretical plates	Not satisfactory	1291	1.03
3	ACN : buffer (pH 5) (20 : 80% v/v)	Completely split peak	Not satisfactory	750	2.12
4	ACN : buffer (pH 6) (20 : 80% v/v)	Completely split peak	Not satisfactory	800	3.89

FIGURE 4: Chromatograms obtained for method development using Acquity BEH C18, (100 × 2.1) mm, 1.7 μ m column.

TABLE 4: Linearity and regression parameters with precision data.

Parameter	Value
Linear range, $\mu\text{g mL}^{-1}$	0.05–300
Limits of quantification, (LOQ), $\mu\text{g mL}^{-1}$	0.05
Limits of detection, (LOD), $\mu\text{g mL}^{-1}$	0.01
Regression equation	
Slope (b)	1989.854
Intercept (a)	1017778.100
Correlation coefficient (r)	0.9999
Standard deviation of b , (S_b)	101.547
Standard deviation of a , (S_a)	16839.785

is an index of accuracy is ≤ 1.5 and is indicative of high accuracy. The calculated percent relative standard deviation (%RSD) can be considered to be satisfactory. The peak area based and retention time based RSD values were < 1 .

3.2.3. Robustness and Ruggedness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. At the deliberate varied chromatographic conditions (flow rate, temperature, and mobile phase composition), the analyte peak % RSD, tailing factor and

theoretical plates remained near to the values obtained under optimum conditions. The RSD values ranged from 0.1 to 1.0% resumes the robustness of the proposed method. In method ruggedness, different columns (same lot) days and analysts ($n = 3$) were performed. The results were summarized in Table 7.

3.2.4. Stability of the Solution. At the specified time interval, % RSD for the peak area obtained from drug solution stability and mobile phase stability were within 1%. This shows no significant change in the elution of the peak and its system suitability criteria (%RSD, tailing factor, theoretical plates). The results also confirmed that the standard solution of drug and mobile phase were stable at least for 24 hours during the assay performance.

3.2.5. Selectivity. Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution, and tablet extract. No peaks were observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts (Figures 6(a) and 6(b)).

4. Application to Tablet Analysis

A 200 $\mu\text{g mL}^{-1}$ solution of tablets was prepared as per “preparation of tablet extracts and assay procedure” and injected in triplicate to the UPLC system. From the mean peak area,

FIGURE 5: Chromatograms obtained for method development using Acquity BEH C18, (100 × 2.1) mm, 1.7 μ m column at different pH conditions.

TABLE 5: Results of accuracy study ($n = 5$).

Concentration of PGZ injected, $\mu\text{g mL}^{-1}$	Intra-day		Inter-day	
	Concentration of PGZ found, $\mu\text{g mL}^{-1}$	RE ^a , %	Concentration of PGZ found, $\mu\text{g mL}^{-1}$	RE, %
100	102.34	2.34	98.47	1.53
200	202.12	1.06	203.21	1.61
300	304.12	1.37	302.45	0.82

^aRelative error.

TABLE 6: Results of precision study.

Intra-day precision ($n = 7$)				
Concentration injected ($\mu\text{g mL}^{-1}$)	Mean area $\pm$ SD	RSD ^a	Mean RT $\pm$ SD	RSD ^b
100	222879 $\pm$ 1648	0.74	2.19 $\pm$ 0.004	0.16
200	502609 $\pm$ 590	0.12	2.19 $\pm$ 0.007	0.32
300	755267 $\pm$ 2276	0.30	2.20 $\pm$ 0.002	0.09
Inter-day precision ($n = 5$)				
Concentration injected ($\mu\text{g mL}^{-1}$)	Mean area $\pm$ SD	RSD ^a	Mean RT $\pm$ SD	RSD ^b
100	223992 $\pm$ 1130	0.50	2.19 $\pm$ 0.024	1.08
200	501345 $\pm$ 3070	0.61	2.19 $\pm$ 0.015	0.67
300	754190 $\pm$ 1555	0.21	2.18 $\pm$ 0.011	0.52

^aRelative standard deviation based on peak area.

^bRelative standard deviation based on retention time.

TABLE 7: Results of method robustness and ruggedness study.

Condition	Modification	Mean peak area ± SD	% RSD	Mean Rt ± SD	% RSD	Theoretical plates ± SD	% RSD	Tailing factor ± SD	% RSD
Actual	—	504198 ± 4130	0.82	2.212 ± 0.001	0.05	3325 ± 8.25	0.25	1.05 ± 0.004	0.39
Temperature	24°C	505867 ± 5852	1.16	2.212 ± 0.001	0.03	3318 ± 6.08	0.18	1.05 ± 0.008	0.78
	26°C	505799 ± 5915	1.17	2.207 ± 0.010	0.45	3435 ± 25.24	0.73	1.05 ± 0.01	1.00
Mobile phase composition (acetonitrile : buffer)	15 : 85	522201 ± 521	0.10	2.213 ± 0.002	0.09	3327 ± 10.12	0.30	1.06 ± 0.006	0.55
	25 : 75	506001 ± 5735	1.13	2.212 ± 0.002	0.09	3329 ± 23.46	0.70	1.05 ± 0.008	0.80
Flow rate	0.22 mL/min	505834 ± 5880	1.16	2.215 ± 0.006	0.27	3331 ± 27.02	0.81	1.05 ± 0.008	0.78
	0.18 mL/min	502734 ± 2170	0.43	2.211 ± 0.001	0.05	3325 ± 15.18	0.46	1.04 ± 0.010	0.92
Wavelength	219 nm	502267 ± 799	0.16	2.211 ± 0.001	0.05	3335 ± 11.93	0.36	1.04 ± 0.009	0.86
	221 nm	502334 ± 521	0.10	2.213 ± 0.003	0.15	3314 ± 5.77	0.17	1.05 ± 0.006	0.55
Analyst, column, day	Analyst-1, column-1, day-1	504198 ± 4130	0.82	2.212 ± 0.001	0.05	3325 ± 8.25	0.25	1.05 ± 0.004	0.39
	Analyst-2, column-2, day-2	502634 ± 295	0.06	2.214 ± 0.004	0.17	3335 ± 31.94	0.96	1.05 ± 0.010	1.09
	Analyst-3, column-3, day-3	505967 ± 5764	1.14	2.214 ± 0.003	0.13	3320 ± 9.02	0.27	1.05 ± 0.010	0.80

FIGURE 6: Chromatograms obtained for placebo blank and tablet extract.

TABLE 8: Results of determination of PGZ in formulations and statistical comparison with the reference method.

Formulation brand name ^a	Nominal amount, mg	% PGZ found ^c $\pm$ SD		<i>t</i> -value	<i>F</i> -value
		Reference method	Proposed method		
Oglo-15 ^a	30.0	99.01 $\pm$ 0.82	98.88 $\pm$ 0.68	0.33	1.73
Neoglit-30 ^b	15.0	101.2 $\pm$ 0.75	99.94 $\pm$ 0.97	1.63	1.40

^aMarketed by Panacea biotec, Mumbai, India.^bMarketed by Novus Life Sciences Private Limited, Mumbai, India.^cMean value of five determinations. Tabulated *t*-value at 95% confidence level is 2.78; tabulated *F*-value at 95% confidence level is 6.39.

the concentration and hence mg/tablet were computed; and the results were compared with those of a reference method [19]. The reference method involved the HPLC analysis with ammonium acetate buffer in acetonitrile and glacial acetic acid (25:25:1), wavelength was set at 269 nm and 4.6-mm, 15-cm; 5- μ m packing L1 column was used. The accuracy and precision of the proposed method were further evaluated by applying Student's *t*-test (<2.7) and variance ratio *F*-test (<6.4), respectively. The *t*- and *F*-values at 95% confidence level did not exceed the tabulated values and this further confirms that there is no significant difference between the reference and proposed methods with respect to accuracy and precision. Table 8 illustrates the results obtained from this study.

5. Recovery Study

A standard addition procedure was followed to evaluate the accuracy of the method. The sample is analysed for the analyte of interest by adding a specified amount of this analyte to the sample, thus increasing its concentration. The percentage recovery of PGZ from pharmaceutical dosage forms ranged from 97.4% to 101.4%. Detailed results presented in Table 9 reveal good accuracy of the proposed method.

6. Stress Study

No degradation products were observed during the stress studies with 1 M HCl, neutral, thermal, and photolytic conditions. Significant degradation was observed with 1 M NaOH and 5% H₂O₂. Figure 7 shows the degradation

chromatograms of PGZ with the corresponding solvent as blank.

7. Conclusion

A reversed phase UPLC method development approach using QbD principles has been described. The Quality by Design project aims to encourage debate about quality in the complete development of the drug in a systematic manner. This paper has reviewed literature on ideas, research, policy, and practice about quality in determination of PGZ as much about what we think as what we know. First, the method goals are clarified based on the process understanding. The experimental design describes the scouting of the key UPLC method components including column, pH, and mobile phase. The interrelationships are studied and the preliminary optimized conditions are obtained for each combination. Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach ensures better design of products with fewer problems in development, reduces the number of trials required for post-market changes, relies more on process, and understanding and mitigation of risk, allows implementation of new technology to improve manufacturing without regulatory scrutiny, enables possible reduction in overall costs of manufacturing resulting in less waste. The validated method is specific, linear, precise, accurate, robust, rugged, and stable for 24 hours and can be applied for the determination in formulated form. The drug is stable in acidic, thermal, photolytic, and hydrolytic conditions and degrades in basic, oxidative conditions. The

TABLE 9: Results of recovery study by standard addition method.

Tablet studied	PGZ $\mu\text{g mL}^{-1}$, tablet	PGZ $\mu\text{g mL}^{-1}$, pure	Total PGZ found, $\mu\text{g mL}^{-1}$	Percent recovery of pure PGZ (% PGZ $\pm$ SD)
Oglo-15	48.58	50	99.56	100.99 ± 0.81
	48.58	150	196.53	98.97 ± 0.48
	48.58	250	299.13	100.18 ± 0.68
Neoglit-30	48.52	50	100.12	101.62 ± 0.85
	48.52	150	195.16	98.31 ± 0.59
	48.52	250	299.60	100.36 ± 0.67

FIGURE 7: Chromatograms obtained for forced degradation.

potential of QbD approach for simultaneous development of multiple methods including impurity methods, assay method, dissolution method, cleaning validation method, and so forth should be implemented.

Acknowledgments

The authors thank Glenmark Pharmaceuticals, Mumbai, India, for gifting pure pioglitazone hydrochloride. They are also thankful to the University of Mysore for providing the permission and facilities to do research work. C. M. Xavier is grateful to Jubilant Life Sciences, Nanjangud, Mysore, for permission to pursue Ph.D. degree programme.

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